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Effects of Endrin on Telencephalic Function in the Pigeon

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The chlorinated hydrocarbon pesticides have important and undesirable toxic effects on many vertebrates. Fish, birds, and farmers have all been poisoned by exposure to these compounds (Radeleff, 1964). There is no certain knowledge of the mechanisms through which these insecticides exert their toxic effects. Endrin, the most toxic of the chlorinated hydrocarbon pesticides (Radeleff, 1964), is thought to act largely on the central nervous system (CNS), since behavioral observations indicate that death from experimental endrin poisoning is associated with convulsions and other so-called CNS signs (Winteringham and Barnes, 1955).

However, a more detailed knowledge of the effects of endrin on the CNS seems desirable. If the compound is simply a convulsant at high doses, with little other effect on the CNS at lower doses, i.e., its toxicity is a threshold phenomenon, then control of these toxic side effects of the drug may be achieved by relatively simple controls of potential exposure. If, on the other hand, endrin is a highly specific and cumulative neurotoxin (Dale *et al.*, 1962) with detectable deleterious effects on the CNS at relatively low doses, then rigid control of endrin usage may be necessary. There have been few direct measurements of the effects of endrin (or related compounds) on the vertebrate brain, and these have not clearly differentiated between the general and specific CNS effects of these insecticides (Gowdey *et al.*, 1952; McNamara and Krop, 1948).

Consequently, the effects of endrin on several neurophysiologic systems were studied. The specific systems selected will be described in the appropriate section of Results. Birds were chosen as the experimental subjects since they are known to be unusually sensitive to endrin, and since pesticide poisoning in birds is a considerable economic problem (James and Davis, 1965; Wurster *et al.*, 1965).

METHODS

Female white carneaux pigeons weighing about 500 g were used in this study. The animals were anesthetized with pentobarbital or urethane slowly injected "to effect" into the brachial vein. Pentobarbital is rapidly metabolized in the bird and was useful in experiments where variations in depth of anesthesia were desired. Urethane in doses from 1.1 to 1.6 g/kg gave an extremely stable level of anesthesia lasting for many hours; it was the most frequently used anesthetic in these experiments. The anesthetized birds were placed in special head holders in a stereotaxic instrument. Recording electrodes were stereotactically placed at selected points in the telencephalon. The most usual loci were the hyperstriatum accessorium, neostriatum caudale, and

the ectostriatum (*Fig. 1*).[1] The recording electrodes were micropipettes of about 1 megohm impedance, filled with 3 *M* NaCl. Recordings were "monopolar" against the frame of the stereotaxic instrument. Pairs of stainless steel microelectrodes were also used; their tip diameters were 1-5 μ and the tip separations were 0.2-0.5 mm. The stimulating electrodes were 3-barrel micropipettes with an overall tip diameter of 40-60 μ, filled with 3 *M* NaCl. Two of the barrels were used for stimulating, the third for recording or as a reserve. The assembly gave stable stimulus thresholds and very localized stimulation. Insulated stainless steel wire pairs were also used. In this case the electrode tip diameters were 0.10-0.15 mm and the tip separation was about 0.25-

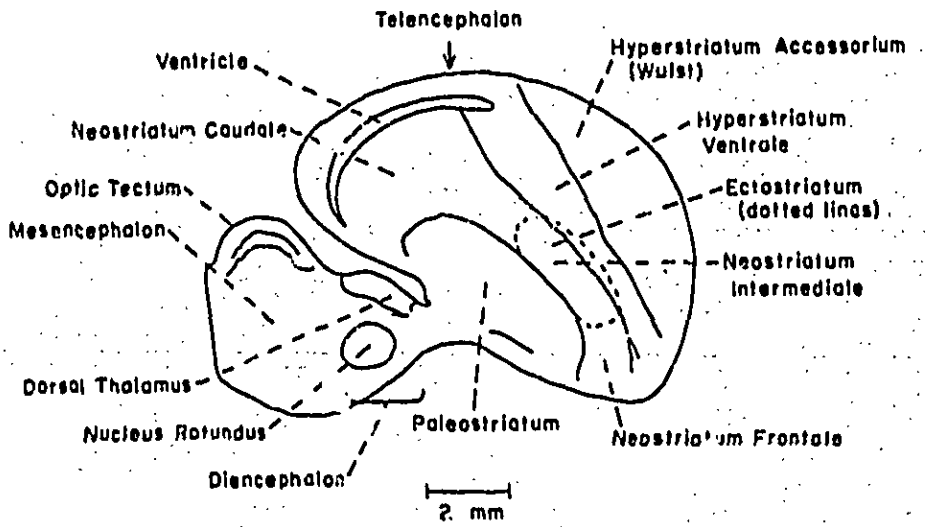

FIG. 1. Avian neuroanatomy: A diagrammatic sagittal section of the pigeon brain. The plane of the section is about 2.5 mm lateral to the midline. The ectostriatum, represented by the dotted lines, is 2.5 mm lateral to the plane of the section. The 2-mm calibration mark is approximate.

0.50 mm. The EEG and evoked potentials were recorded on ink-writers and film, respectively. The amplifying, stimulating, and recording systems were conventional, as were the neurohistologic procedures used to confirm the electrode positions.

Purified endrin was obtained commercially. The compound was administered via a cannula in the brachial vein. It was dissolved either in ethyl alcohol or in dimethyl sulfoxide. Both solvents were without detectable effects on the neuroelectric phenomena studied, in the quantities injected.

RESULTS

Endrin Toxicity

The first step in this study was the determination of an approximate LD_{50} for endrin in the unanesthetized bird to serve as a guide to dosage in the anesthetized preparation. Twelve birds were used. The LD_{50} for intravenous endrin was found to

[1] The avian neuroanatomical terminology used is that of Kappers *et al.* (1960).

be approximately 1.5 mg/kg. The slope of the dose-response curve was very steep; 1.2 mg/kg was an LD_0 whereas 2.0 mg/kg approximated an LD_{100}. Severely poisoned birds showed tonic-clonic convulsions which began 20-30 minutes after endrin injection. The seizure pattern was similar to that seen after pentylenetetrazole poisoning and has been described previously (Winteringham and Barnes, 1955). These convulsions were usually lethal within about an hour of the initial seizure. If the birds survived longer than this, they generally recovered completely. The surviving birds showed no grossly detectable symptomatology beyond 4 hours after drug injection. In this respect acute and chronic endrin poisoning differ considerably.

Effects of Endrin on EEG in Anesthetized Pigeons

The spontaneous electrical activity (EEG) recorded from the surface of the avian cerebrum under normal conditions is a mixture of high and low frequency waves looking very much like the neocortical EEG of mammals (Bremer *et al.*, 1939; Ookawa and Gotoh, 1965). Recordings from most of the subsurface structures in the avian telencephalon are similar except for those from the neostriatum intermediale-ectostriatum-anterior neostriatum caudale complex. These structures were characterized by a very large-amplitude rhythmic spikelike wave form. The duration of the waves was 50-200 msec, and their average frequency was about 3/sec (Revzin, 1965).

At endrin dosages of 1.2 mg/kg, an increase in wave frequency was seen, together with an increase in the high-frequency components of the surface EEG (Fig. 2). With doses of endrin above 3.0 mg/kg seizure discharges were seen in all parts of the telencephalon (Fig. 2). The largest amplitude discharges were seen in the ectostriatum, and often were seen here before electrographic changes were seen in other structures. Furthermore, seizure activity was frequently more prolonged in the ectostriatum than in the other CNS structures. The total duration of a seizure ranged from 3 seconds to about a minute. The seizure was followed by a quiet period of 5-15 minutes, after which another seizure would occur. However, the inter- and intra-animal variability in the seizure patterns was very great, so that little meaning can now be attached to the duration of the parts of the seizure cycle. No overt motor phenomena were seen at any time during or after these seizures. Thus, no effective convulsive activity was propagated from the discharging telencephalic centers into the spinal cord or medulla, and, therefore, in contrast to strychnine, endrin had little direct convulsive activity on neuronal structures in the spinal cord or medulla. Since motor signs do appear in normal unanesthetized animals after endrin poisoning, it is assumed that the anesthesia effectively blocked propagation of seizure discharges from the telencephalon to the lower motor centers.

The electrographic effects of endrin are completely blocked by sufficient doses of pentobarbital (Fig. 2C, Fig. 3C).

Endrin Effects on Reticular Stimulation

Stimulation of the ascending reticular activating system (ARAS) in pigeons causes a "desynchronization" of the EEG in telencephalic structures (Revzin, 1965) (Fig. 3). This desynchronization is similar to that seen in mammals following ARAS activation (Moruzzi and Magoun, 1949). The effects of ARAS stimulation differ somewhat in

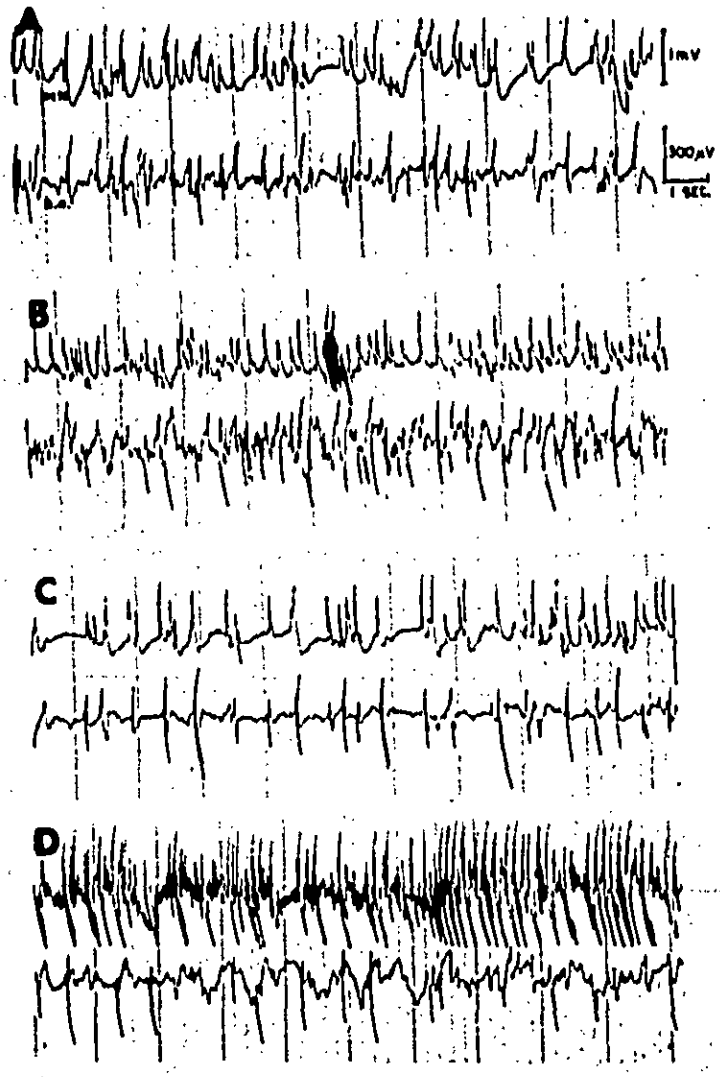

FIG. 2 (A) Normal recordings from the ectostriatum (*ecto*) and hyperstriatum accessorium (*h.a.*) in a pigeon anesthetized with urethane. Note the tendency for the spikelike wave forms to form clusters and the lack of temporal relationship between "spiking" activity in the two brain areas. (B) Taken 30 minutes after 2 mg/kg of endrin. Increased spiking and high frequency activity in both leads, and "seizure" activity in the hyperstriatum. (C) Taken 20 minutes after B and 10 minutes after 15 mg/kg of pentobarbital. The anesthetic effectively suppresses the endrin effect. (D) One minute after record C another 1 mg/kg of endrin was given. This record was taken 30 minutes later. Full electrographic convulsions are seen in the ectostriatum, but not in the hyperstriatum. Propagation of the seizure from the ectostriatum did occur in this animal, but not frequently.

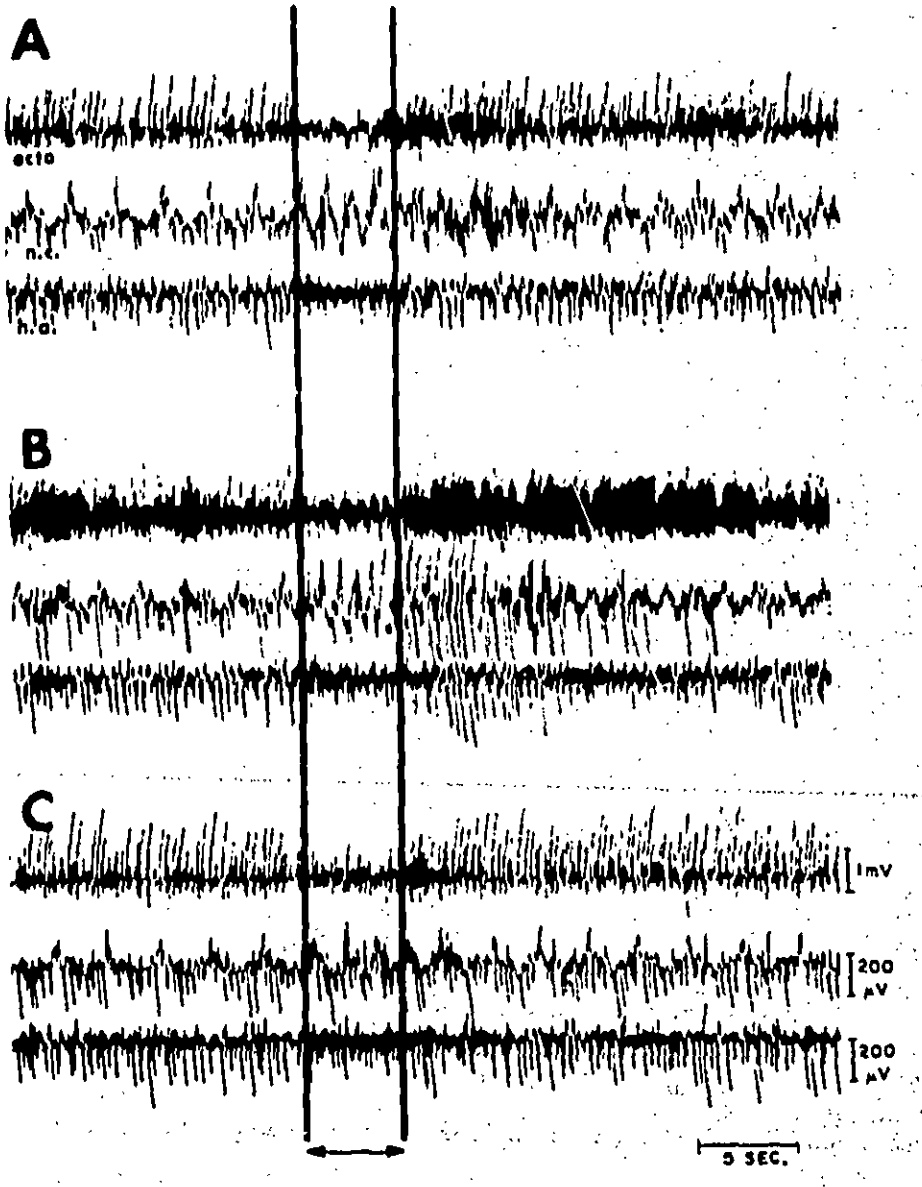

FIG. 3. (A) Control record taken in a pigeon anesthetized with urethane. The anesthesia was light so more "spiking" is seen here than in Fig. 2. The nucleus pontis oralis was stimulated at 100/sec for 5 seconds, indicated by the vertical lines. Note "desynchronization," marked in the ectostriatum (*ecto*) and the hyperstriatum accessorium (*h.a.*), but not very prominent in the neostriatum caudale (*n.c.*). (B) Same, 40 minutes after endrin, 3 mg/kg. Seizure activity was limited to the ectostriatum at this time. Reticular stimulation caused a suppression of seizure activity and a poststimulus rebound effect. (C) Same, 20 minutes after pentobarbital, 20 mg/kg. Record is near normal.

the various telencephalic nuclei (Revzin, 1965), but these differences are not relevant to the present discussion.

Endrin in subconvulsive doses has little or no effect upon ARAS-induced desynchronization of the avian EEG. Endrin given in convulsive doses has a diphasic action upon the effects of ARAS stimulation. Within the first 10-15 minutes after endrin administration, the threshold for desynchronization rises by about 25%. Thresholds return to control levels about 20 minutes after endrin injection. Then, as fast activity and seizures develop, the thresholds drop to 85-90% of the control values. This occurs

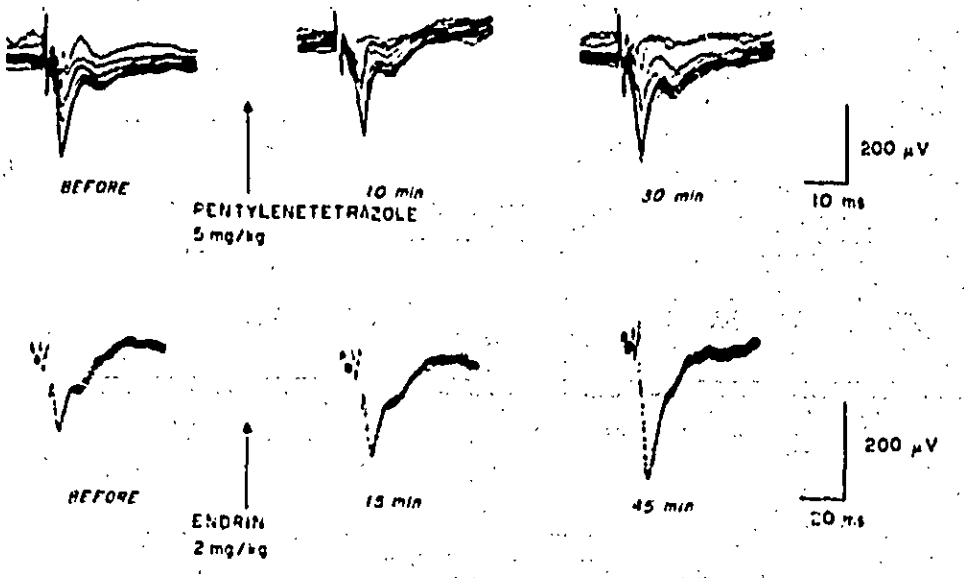

FIG. 4. The lower line shows rotundo-ectostriatal-evoked potentials (REP) before, 15, and 45 minutes after 3 mg/kg of endrin. The upper line shows REP's before and after 60 mg/kg of pentylenetetrazole. The endrin dose used was not quite sufficient to induce seizure activity in this bird; the dose of pentylenetetrazole given was about three times that needed to induce seizures. The records are taken from different experiments. The records in the upper series are photographs of 10 superimposed sweeps of the oscilloscope beam; the lower series are photographs of a computer read-out.

about 30 minutes after endrin administration. The thresholds remained slightly below control levels for the duration of the experiments (2-4 hours). These thresholds were obtained during the interseizure intervals or while fast activity was increasing just prior to a seizure. During the seizure, and for a short time thereafter, ARAS stimulation was frequently without detectable effect on electrical activity. However, reduction in the amplitude of seizure discharge or an inhibition of the discharges was often seen during ARAS stimulation (Fig. 3B).

Endrin Effects on Evoked Potentials in the Avian Visual Projection System

We are investigating a projection pathway by which visual impulses are relayed into the avian telencephalon. One of the stages in this relay system is a monosynaptic projection from the nucleus rotundus, the largest and best differentiated nucleus in

the avian diencephalon, to another large nucleus, the ectostriatum, in the telencephalon (Fig. 1). After electrical stimulation of the nucleus rotundus, a complex and very large evoked potential, the rotundo-ectostriatal potential (REP), is produced in the ectostriatum. The REP has two components, an early and usually small di- or triphasic spike with a peak latency of 2-3 msec, and a later, longer duration and larger negative-positive wave with a peak latency of 5-7 msec. The early response is a "radiation response" representing the activity in the rotundo-ectostriatal nerve fibers. The later wave represents the mean response of ectostriatal neurones to the incoming nerve volley. Since the nuclei are large and accessible, and the potentials large and stable, the rotundo-ectostriatal system is very useful for neuropharmacologic analyses.

Administration of endrin causes a detectable increase in the amplitude of the late phase of the REP at dosages of 0.5-1.0 mg/kg (i.e., about 30% of the ED_{50} for seizure activity). At doses of endrin near seizure levels in the anesthetized animal, about 3 mg/kg, the REP may reach 2.5 times the control amplitudes (Fig. 4). The amplitude of the early phase of the REP (the radiation response) is not affected by endrin (Fig. 4).

The effects of pentylenetetrazole were also studied. This compound, like endrin, had no effect on the radiation response, and also caused an increase in the later components of the REP. This latter effect was small and inconsistent, the increase never exceeding 25% (Fig. 4). Furthermore, the dose of pentylenetetrazole required to show this REP increase was 2-3 times the dose required to induce electrographic seizures in the ectostriatum and other forebrain structures. Thus, the REP facilitation induced by pentylenetetrazole differs considerably from that induced by endrin.

DISCUSSION

Administration of adequate doses of endrin to birds will induce electrographic seizure activity in telencephalic structures and will facilitate certain evoked potentials. These findings confirm the previous evidence that endrin and, probably, the other chlorinated hydrocarbon pesticides are neurotoxins in vertebrates. The present results permit some further conclusions.

An objective of this investigation was to determine whether endrin shows a selective pattern of toxicity or is a "general" CNS stimulant. There is evidence for both possibilities. At doses in excess of 4 mg/kg in the anesthetized animal, endrin will induce seizures in all telencephalic nuclei examined. There is no clear evidence of propagation from any one center to the others at these doses. Thus, endrin in sufficient dosage may be considered a nonspecific or general CNS convulsant, analogous perhaps to strychnine or pentylenetetrazole.

However, in lower doses the convulsions seen are not identical in all nuclei. The amplitude of the seizure discharges is much larger in the ectostriatum and adjacent portions of the neostriatum than in the hyperstriatum, the paleostriatum, or in the surface leads. Furthermore, both the buildup of high-frequency activity which precedes seizures and the initiation of seizure discharges often occur in the ectostriatum prior to their appearance in other nuclei, although the opposite was not seen. In addition, the duration of the seizure discharges tends to be longer in the ectostriatum than in other structures. In some experiments, indeed, seizure activity was seen only in the ectostriatum (Fig. 3). There is too much variability in these records to justify

a definitive conclusion, but the foregoing certainly suggests that the ectostriatum is more sensitive to endrin than the other forebrain structures (Fig. 2). This suggestion is reinforced by the effects of endrin on the rotundo-ectostriatal evoked potential. The compound enhances the postsynaptic component of the evoked potential in doses too low to affect the other electrophysiologic phenomena studied. This effect of endrin on evoked potentials is not duplicated by pentylenetetrazole.

Furthermore, endrin had no effect on several of the phenomena studied. Thus, even administration of very large doses of endrin did not affect the "radiation response" component of the rotundo-ectostriatal evoked potentials, which suggests that endrin had little effect on the excitability of the cells in the nucleus rotundus. Also, endrin had relatively little effect on the EEG response to ARAS stimulation. Some small threshold changes were produced by endrin. However, the qualitative effects of ARAS stimulation were frequently not affected even at the height of a seizure (Fig. 3). Thus, the drug probably did not affect any component of the polysynaptic ARAS-telencephalon projection. Although the presence of anesthetics complicates the interpretation somewhat, the absence of effects on the ARAS and the nucleus rotundus further strengthens the suggestion that endrin shows selective toxicity within the vertebrate CNS.

The facilitation of the REP seen after subconvulsive doses of endrin suggests that the compound affects the ectostriatum in birds at doses below those needed to produce overt behavioral disturbances. Unpublished work in this laboratory has established that the ectostriatum is a visual projection area probably analogous in function to the visual projection areas in the mammalian telencephalon. Consequently, it is probable that endrin will produce visual deficits in birds through its action on the ectostriatum. Furthermore, the doses required to produce such perceptual deficits will be materially lower than those necessary to produce grossly observable behavioral changes. Indeed, James and Davis (1965) have recently reported visual perceptual deficits in birds poisoned with very low levels of DDT. Birds are, of course, highly dependent on vision, and the visual deficits induced by poisoning with chlorinated hydrocarbon insecticides would probably reduce the bird's ability to avoid predators and compete for food. Thus, the selective toxicity of endrin on the ectostriatum may explain in part the unusual sensitivity of birds toward endrin and DDT. The practical consequences of this finding may be considerable. It would seem from the above discussion that chlorinated hydrocarbon pesticides may affect bird populations at doses well below those required for grossly detectable behavioral changes. Furthermore, the compound has a long half-life in the body and can accumulate even when environmental levels are low (Dale *et al.*, 1962). This suggests that in situations where the loss of bird populations is ecologically or economically undesirable, the use of chlorinated hydrocarbon pesticides should be reviewed very carefully. Although similar selective toxicity patterns have not been conclusively demonstrated in mammals, there is evidence of their occurrence in animals (Philips and Gilman, 1946) and in man.[2] Thus, much further research into the mechanisms of pesticide toxicity in the vertebrate CNS seems indicated.

[2] Personal communication from Dr. David R. Metcalf, University of Colorado, Denver, Colorado.

SUMMARY

Intravenous injection of endrin in the anesthetized pigeon induced a number of changes in telencephalic neuronal function. Dosages of 4 mg/kg or more caused seizure activity throughout the telencephalon. At 2-3 mg/kg, endrin caused seizure activity largely limited to the ectostriatum, a telencephalic visual projection area. At 0.5-2 mg/kg, endrin caused a specific increase of potentials evoked in the ectostriatum by stimulation of the nucleus rotundus, a diencephalic visual projection area. Reticular formation functions tested were little affected by endrin at any dosage tested. It is suggested that relatively low brain levels of endrin may impair visual function in birds, and that this visual impairment could be a major factor underlying the well known sensitivity of birds toward the chlorinated hydrocarbon pesticides.

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